Treponemal Serologic Tests

Experiences of the Bacteriology Laboratory, California State Department of Public Health

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• **In a study of the relationship of clinical impression regarding syphilis and age, sex and pregnancy status to treponemal serologic test reactivity, it was noted that in diagnostic "problem cases" the standard lipid serologic test titers did not differentiate between syphilitic and biologic false positive reactors. Preliminary data indicated that heroin addiction may be a source of biologic false reactions and that pregnant women with standard serologic test reactivity have a lower treponemal reactivity rate than other women with lipid serologic reactivity.**

NOTWITHSTANDING the general downward trend in reported cases of syphilis during the past decade, this infection represents a continuing problem to clinicians, epidemiologists and microbiologists.

An ever present problem to clinicians and laboratorians is the biologic false positive (BFP) reaction with the standard lipid tests for syphilis in patients in whom there is no historical, clinical or epidemiological evidence of syphilitic infection. Moore and Mohr[6] expressed the belief that in certain population groups at least half the persons with serologic reaction in mass blood-testing programs do not have syphilis. Although these reactors represent a small fraction of the total number of persons tested in such a program, they present a very real problem to physicians.

Following the description of the Treponema pallidum immobilization (TPI) test in 1949 by Nelson and Mayer,[7] serologic procedures employing treponemal antigens have become available which are considered to possess a high level of sensitivity and specificity in the treponematoses. In a collation of results of the clinical significance of the TPI on some 10,035 sera and 323 specimens of spinal fluid, Nielsen and Reyn[8] reported that the general sensitivity of the TPI test appears to be high except in early syphilis; the TPI result was positive in about 60 per cent of the cases of primary syphilis studied, in about 90 per cent of the secondary cases and in nearly 100 per cent of the cases of longer duration. These investigators also reported a high level of specificity.

Although his study series was smaller, Zellerman[10] noted false treponemal test results in only 0.3 per cent of the cases studied.

The Treponema pallidum complement fixation test (TPCF) described by Portnoy[9] in 1955 is reported to be more sensitive in early syphilis than is the TPI test; and the false-positive incidence is reported to be less than 1 per cent.[4]

The treponemal testing program of the United States Public Health Service has provided a much needed opportunity for an evaluation of the reactivity patterns of some of the standard serologic tests for syphilis in diagnostic problem cases. It has been repeatedly shown that the standard serologic tests for syphilis demonstrate satisfactory sensitivity in cases of syphilis and a high level of specificity in selected nonsyphilitic persons. However, little has been published concerning the reactivity level of these tests in diagnostic problem cases. Indeed, until the advent of the treponemal tests, it was extremely difficult to establish a standard of reference whereby the serologic tests employing lipid antigens could be evaluated.

Data presented in this paper concern the relationship of treponemal tests and the reactivity level of standard serologic tests employing lipid antigens. These tests were performed respectively in the Venereal Disease Research Laboratory (VDRL) at Chamblee, Georgia, and the California State Division of Laboratories. The term *lipid test* is used to describe standard serologic tests employing either crude lipoidal or cardiolipin-lecithin antigens. The serologic tests selected were the VDRL slide test and the Kolmer complement fixation test. Both of these tests employ cardiolipin-lecithin antigens. The standard Kolmer test employs an antigen in which the ratio of lecithin to cardiolipin is 1.66 to 1. In addition to this antigen, the Kolmer test was also performed with two less sensitive antigens. One of these was the crude lipoidal antigen known as "New Improved Kolmer Antigen,"[5] which is still used in a number of laboratories, and the other was a cardiolipin-lecithin antigen with a ratio of lecithin to cardiolipin

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TABLE 1.—*Clinical Impression and Treponemal Reactivity on 1,290 Specimens Submitted for TPI Testing*

Clinical Impression	Number	Per Cent	TPI Reactivity			
			Number		Per Cent	
			Reactive	Nonreactive	Reactive	Nonreactive
Syphilis	224	17	156	68	70	30
BFP	941	73	415	526	44	56
Not stated	125	10	67	58	54	46
Total	1,290	100	638	652	49	51

Abbreviations: TPI = Treponema pallidum immobilization; BFP = Biologic false positive.

TABLE 2.—*TPI Reactivity of 1,286 Patients by Age and Sex*

Age Group—Years	Males			Females		
	Total No.	TPI Reactive		Total No.	TPI Reactive	
		No.	Per Cent		No.	Per Cent
<16	10	4	40	6	0	0
16 to 45	316	152	48	558	238	43
46>	192	116	60	204	128	63
All ages	518	272	53	768	366	47

TPI = Treponema pallidum immobilization.

TABLE 3.—*TPI Reactivity in 768 Women According to Pregnancy Status*

Pregnancy Status	Total No.	TPI Reactive		TPI Nonreactive	
		No.	Per Cent	No.	Per Cent
Pregnant	135	38	28	97	72
Pregnancy not indicated	633	328	52	305	48

TPI = Treponema pallidum immobilization.

of over 11 to 1 and a sensitivity about equal to the new improved Kolmer antigen. This formulation of Allen and Mason[2] is now used in the National Health Department Laboratories in Canada. However, in this study it has been used only on an experimental basis and individual test results have not been reported to physicians.

The USPHS Venereal Disease Research Laboratory through State and Territorial Health Departments has offered a nationwide treponemal testing service since January 1955. The criteria established by the USPHS required that specimens be submitted only on diagnostic problem cases in which there was a possible discrepancy between clinical opinion and serologic test results. All patients with reaction to lipid tests, except pregnant women, were to be followed with these tests for a minimum of three months before specimens were sent for treponemal testing. Physicians were further required to submit a clinical data sheet for each patient. The information requested included: History of syphilis in the patient or his family, evidence of other treponemal or other venereal disease, evidence of certain diseases thought to cause biologic false positive reactions in the standard tests, record of previous standard test results, the physician's clinical impression of the significance of these results—that is, whether he thought them due to biologic false reactivity or to syphilis. No data were requested regarding pregnancy or drug addiction. Requirements were established regarding collection and submission of specimens in an effort to insure reliability of test results.

In California whole blood specimens accompanied by the clinical data sheet and a syphilis serologic test request form were submitted to the State Division of Laboratories by private physicians, local health departments and venereal disease clinics. A portion of serum accompanied by the clinical data sheet was sent to the Venereal Disease Research Laboratory for treponemal testing. When there was an adequate amount of serum, a portion was reserved for testing in the California State Laboratory with the standard lipid tests. The procedures employed in performing these tests are outlined in the 1955 Manual of Serologic Tests for Syphilis.[1] Data from the history sheet, correspondence and the state serology form as well as standard and treponemal test results were entered on marginal punch cards. This procedure afforded an opportunity for comparison of lipid and treponemal test reactivity on "problem" sera in California.

From January 1955 to January 1957, 1,290 sera were tested in the Venereal Disease Research Laboratory by the TPI and in the California State Laboratory with the lipid tests indicated. Because of serum limitations, all four standard serologic tests were not

TABLE 4.—*Agreement of Individual Lipid Tests with Treponemal Test*

STS Test*	Number Sera Tested	Per Cent Agreement of STS with Treponemal Test: Treponemal Reactive Sera	Treponemal Nonreactive Sera	Overall Agreement with Treponemal Test
VDRL slide	1,576	93	42	68
Kolmer's cardiolipin (1)	1,530	86	46	66
Kolmer lipoidal (5)	1,320	55	80	68
Kolmer Canadian (2)	347	59	83	73

*Numbers in parentheses indicate reference citation.
Abbreviations: STS = Standard serologic test; VDRL = Venereal Disease Research Laboratory test.

performed on every specimen. Early in 1957, the testing procedure at the Venereal Disease Research Laboratory was changed from the TPI to the Treponema pallidum complement fixation (TPCF) test. From January through March 1957, 290 additional sera were tested in the two laboratories. The comparison of STS reactivity with treponemal test reactivity was essentially the same for the TPI and the TPCF. Therefore, in many instances in this paper, no distinction will be made as to which treponemal test was performed. For purposes of comparison and simplification, weakly reactive and reactive qualitative test results are grouped as reactive in both the standard and treponemal tests.

Treponemal Reactivity by Clinical Impression (Table 1)

A physician submitting a specimen was asked to state if in his opinion the serologic test for syphilis (STS) reactivity represented syphilis or a biologic false positive (BFP) reaction. A clinical impression of syphilis was indicated for 17 per cent of the patients, an impression of BFP for 73 per cent, and no impression was stated for 10 per cent. Of the 1,290 specimens submitted for TPI testing, 49 per cent were TPI test reactors and 51 per cent were TPI nonreactors. The TPI test showed reaction in 70 per cent of the patients with a physician's impression of syphilis, in 44 per cent of the patients believed by the physician to have BFP, and in 54 per cent of the patients about whom no impression was given.

Treponemal Reactivity by Age, Sex, and Pregnancy Status (Tables 2 and 3)

There were 1,286 specimens submitted for TPI testing upon which data were given regarding the patient's sex and age. Five hundred and eighteen or 40 per cent of these specimens were from males and 768 from females. The number of specimens in the age group under 16 years was considered too small to permit meaningful analysis regarding TPI reactivity in this group. In the age group of 46 years and over, 60 per cent of men and 63 per cent of women had TPI reactivity. In the 16-45 year group, 48 per cent of males and 43 per cent of women were reactive.

Although the history form did not ask for a statement of pregnancy status, the physicians submitting the specimens noted pregnancy in 135 women, all of whom were in the 16 to 45 age group. Data for TPI reactivity according to pregnancy status are given in Table 3. Of the 135 women known to be pregnant, only 38 (28 per cent) were TPI reactive. In all other women, TPI reactivity was 52 per cent, which closely approximates the 53 per cent treponemal reactivity of all males.

Treponemal Reactivity in Other Conditions

Because of a lack of consistency in the data submitted on the history form employed in this study, an evaluation of treponemal test reactivity in conditions commonly thought to cause false positive STS results was not made. However, it is of interest that 25 heroin addicts who were presumably nonsyphilitic but had standard test reaction, had only half the incidence of treponemal reactivity observed with other patients.

Comparison of STS and Treponemal Reactivity (Table 4)

In this group of sera from problem patients, the VDRL slide test results agreed best with the treponemal reactive results and poorest with the treponemal nonreactivity. Of 1,576 sera tested by the VDRL, 93 per cent were reactive with the treponemal reactive sera but only 42 per cent were nonreactive in the treponemal nonreactive sera. The cardiolipin Kolmer test was the next most reactive test. In results on 1,530 sera tested, 86 per cent agreed with the treponemal reactors and 46 per cent agreed with the treponemal nonreactors. In 1,320 sera, the lipoidal Kolmer test was reactive in only 55 per cent of the treponemal reactive specimens but was nonreactive in 80 per cent of the treponemal nonreactive sera. Testing procedures were not started with the Canadian cardiolipin Kolmer antigen until November 1956. From that date to May of 1957, 347 sera were tested. The Canadian Kolmer test was reactive in 59 per cent of the treponemal reactors tested and was nonreactive in 83 per cent of the treponemal nonreactive sera, which closely approximates the reactivity agreement of the lipoidal Kolmer test.

Overall agreement between the treponemal tests and each of these lipid tests was between 66 and 73 per cent.

Whenever possible, several STS were performed on each specimen submitted for treponemal testing to determine if a battery of tests would be helpful in solving these serologic problems. There were 1,302 specimens upon which it was possible to perform the VDRL slide test and the Kolmer test with cardiolipin and lipoidal antigen (Table 5). In 78 per cent of the sera that were reactive to all three STS, the TPI was also reactive. The incidence of TPI reactivity dropped to 50 per cent on sera reactive to only two STS, and to 33 per cent on sera reactive to only one STS. In 89 per cent of the sera where all three STS were nonreactive, the TPI was also nonreactive.

TABLE 5.—*Lipid and Treponemal Test Reactivity on 1,302 Specimens*

STS*	Total Specimens	Treponemal Test: Reactive No.	Reactive Per Cent	Nonreactive No.	Nonreactive Per Cent
Reactive in 3 STS	426	330	78	96	22
Reactive in 2 STS Nonreactive in 1 STS	400	198	50	202	50
Reactive in 1 STS Nonreactive in 2 STS	252	83	33	169	67
Nonreactive in 3 STS	224	25	11	199	89

*Tests employed: Venereal Disease Research Laboratory slide; Kolmer with cardiolipin and lipoidal antigens.

Relationship Between STS Titer and Treponemal Reactivity

If the available specimen was large enough, quantitative tests were performed in an effort to determine the relationship of STS titer to treponemal reactivity on sera from patients presenting diagnostic problems. In sera reactive in the VDRL slide test (Chart 1), there was no difference in titer between presumably syphilitic sera (TPI reactors) and presumably biologic false positive sera (TPI nonreactors). Ninety per cent of the reactions occurred at dilutions lower than 1:8. Although not shown in the chart, the distribution of titers obtained in the Kolmer test with lipoidal and with Canadian antigen very closely paralleled that obtained in the VDRL slide test. The charted curve of the standard cardiolipin Kolmer antigen test results was similar to that of the VDRL, but it was reactive at a somewhat higher titer, some 40 per cent of the sera showing reaction in dilutions of 1:8 or higher.

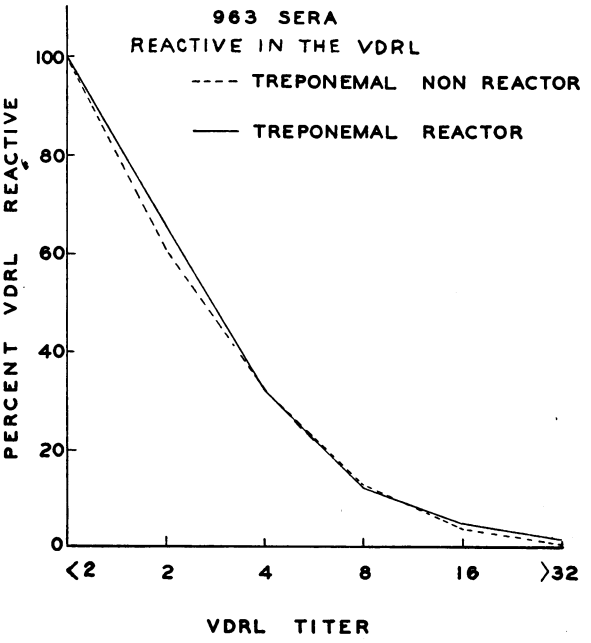

Chart 1.—Showing lack of agreement between VDRL test (Venereal Disease Research Laboratory) and treponemal immobilization test.

DISCUSSION

The correlation between clinical opinion and treponemal reactivity reported here is similar to that previously reported by Bossak and associates[3] for the United States as a whole. In over 50 per cent of the cases in which the physician considered the STS reaction a false positive one, the TPI bore out the clinical impression. However, over 40 per cent of the presumably false reacting patients were treponemal reactive.

The data presented in Chart 1 clearly indicate that in the individual patient it is impossible to predict treponemal reactivity from standard serologic test reactivity. In patients presenting diagnostic problems, there was no correlation between treponemal reactivity and the amount of reagin present as determined by quantitative STS. In this series of tests, several specimens with STS titers reaching 1:5,000 were treponemal nonreactive. However, when a battery of STS was performed, the greater the number of reactive lipid tests, the greater the chance that the treponemal test would also be reactive. Conversely, the greater the number of nonreactive lipid tests, the greater the chance that the treponemal test would also be nonreactive. While statistical data cannot apply to an individual patient, the finding of lipid reactivity in two or more STS set at different levels of reactivity may offer a clue for the deeper probing into the medical, physical and social history of patients who have such reactivity. It would seem equally important to do further studies of a patient with discrepancy between results of serologic tests. An appreciable number of patients showing serologic

reactivity over a period of three months or more in only one or two of several STS, were reactive to the treponemal tests. Thus, it would appear that any presumably false serologic reaction which cannot be definitely attributed to some other condition and which persists for a period of at least three months should be checked by one or more of the available treponemal procedures.

The finding of a significantly lower level of TPI reactivity in pregnant women than in all other women tested is of interest. However, it must be pointed out that a different basis of selection was employed for the two groups. Pregnant women were not required to be followed for three months with the standard tests as were all other patients. It is probable that some of the treponemal nonreactors in the pregnant group would not have been included in the study had these women been followed for three months with the lipid tests as were other patients in this study. Nonetheless, there is evidence that pregnancy is of itself a cause of biologic false reaction of the acute type. It is hoped that studies now in progress will provide additional information on both pregnancy and heroin addiction as possible causes of false positive reactions in the standard tests.

Although the VDRL test results gave the poorest agreement with treponemal test nonreactors in this series of problem specimens it should not be inferred that this test is unsatisfactory for routine use. Experience is quite to the contrary. Routine serologic tests are not specific for syphilis but react in nearly 100 per cent of late primary, secondary and early latent cases. In determining test reactivity levels, serologists reporting on studies must achieve a nice balance between ability to react in cases of luetic infection and inability to react in serum from presumably nonsyphilitic individuals. It has been a general experience that as test sensitivity increases, specificity decreases. Regardless of the sensitivity level, serologic tests for syphilis must be subject to control of reproducibility of test results at the operating level and not be too cumbersome to perform. Of tests in common use, the VDRL seems best to meet the needs of clinicians and of laboratories in these respects.

Perhaps a word is needed about the validity of data involving the smaller number of tests employing the Canadian antigen formulation in the Kolmer test. When other STS antigens were evaluated in terms of TPI results at the same stage of experience, there was no decided deviation from that herein reported. We feel the data given are a reliable index of future experience.

In the present series of patients, there was no known bias in selection according to racial or socioeconomic status. The patients would appear to represent a cross-section of the population of California. However, data are needed to evaluate the influence of these factors in determining treponemal reactivity.

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